

**SPECIAL SECTION:
GRA N₂O CHAMBER METHODOLOGY GUIDELINES**

Global Research Alliance N₂O chamber methodology guidelines: Introduction, with health and safety considerations

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Abstract

Non-steady-state (NSS) chamber techniques have been used for decades to measure nitrous oxide (N₂O) fluxes from agricultural soils. These techniques are widely used because they are relatively inexpensive, easy to adopt, versatile, and adaptable to varying conditions. Much of our current understanding of the drivers of N₂O emissions is based on studies using NSS chambers. These chamber techniques require decisions regarding multiple methodological aspects (e.g., chamber materials and geometry, deployment, sample analysis, and data and statistical analysis), each of which may significantly affect the results. Variation in methodological details can lead to challenges in comparing results between studies and assessment of reliability and uncertainty. Therefore, the New Zealand Government, in support of the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases (GRA), funded two international projects to, first, develop standardized guidelines on the use of NSS chamber techniques and, second, refine them based on the most up to date knowledge and methods. This introductory paper summarizes a collection of papers that represent the revised guidelines. Each article summarizes existing knowledge and provides guidance and minimum requirements on chamber design, deployment, sample collection, storage and analysis, automated chambers, flux calculations, statistical analysis, emission factor estimation and data reporting, modeling, and “gap-filling” approaches. The minimum requirements are not meant to be highly prescriptive but instead provide researchers with clear direction on best practices and factors that need to be considered. Health and safety considerations of NSS chamber techniques are also provided with this introductory paper.

Abbreviations: FC, flux calculation; GC, gas chromatography; GRA, Global Research Alliance on Agricultural Greenhouse Gases; H&S, health and safety; NSS, non-steady state.

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1 | INTRODUCTION

For many decades, researchers have used static or “non-steady-state” (NSS) chamber techniques to measure nitrous oxide (N_2O) fluxes from agricultural soils. Non-steady-state chambers measure N_2O emissions by placing an open-bottomed chamber on the soil surface and measuring the accumulation of N_2O in the headspace of the chamber over a short time period. Daily N_2O fluxes have typically been estimated from chamber measurements taken once on a given sampling day, whereas spatially and temporally integrated cumulative emissions are based on daily flux measurements taken from a given number of replicate chambers, at a given sampling frequency, over the entire experimental period (typically covering a few months to a full year).

Although micrometeorological methods for determining soil-to-atmosphere N_2O fluxes have also been available for some decades (Flesch et al., 2018; Hargreaves, 1994; Wagner-Riddle, Park, & Thurtell, 2006), NSS chambers are still more commonly used as they are relatively inexpensive, easy to adopt, versatile, and adaptable to varying field conditions. Rochette (2011) reported that 95% of published N_2O emission experiments used NSS chambers. Consequently, much of the understanding of the drivers of N_2O emissions, the efficacy of mitigation practices, as well as assessments of national and global agricultural N_2O emission inventories are based on NSS chamber measurements (Chadwick et al., 2018; David, Lemke, Helgason, & Farrell, 2018; Luo, Saggar, van der Weerden, & de Klein, 2019; Rochette & Ericksen-Hamel, 2008; van der Weerden et al., 2020).

Chamber methodologies adopted by researchers can vary due to differences in the aims of the measurements (e.g., exploring trends across landscape transects, evaluating differences between treatments, or emission factor measurements for inventory purposes). However, even methodologies for projects with the same aim can vary due to differences in the physical design of chambers, the methods and frequency of their deployment in the field, the type of analyzer used to quantify N_2O concentrations in head space samples, and data processing techniques used to estimate hourly, daily, and cumulative fluxes. As a result, comparisons of results between studies, as well as assessments of their reliability and uncertainty, can be challenging. Rochette and Ericksen-Hamel (2008) evaluated various aspects of chamber methodologies that were used in 365 global N_2O studies and concluded that, based on the information provided in the published papers, >50% of these studies were of a “poor” or “very poor” quality when judged against certain “robustness” criteria.

The global science community therefore recognized the need for standardized guidelines on the use of chambers

Core Ideas

- Standardized guidelines on the use of N_2O chamber techniques have been revised.
- This article summarizes a collection papers that represent the revised guidelines.
- Guidance on minimizing health and safety risks of chamber techniques is provided.

for determination of N_2O fluxes and associated data reporting. The New Zealand Government agreed to fund an international project to develop such guidelines in support of the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases (GRA). The GRA is an alliance between countries to find ways to grow more food without growing greenhouse gas emissions (<https://globalresearchalliance.org/about/>). Initial versions of the guidelines, which were self-published by the GRA in 2012 and slightly revised in 2015, summarized existing knowledge and provided guidance and recommendations, with each chapter focusing on one key aspect of chamber methodologies, including design; deployment protocol; air sample collection, storage, and sample analysis; data analysis; and experimental data reporting. It also included additional chapters on automated chamber systems and health and safety (H&S) considerations. Since the initial guidelines were first published, the global research community has continued to address the challenges of N_2O chamber methodologies, and many aspects have been refined. Therefore, the GRA recognized the need to update the guidelines with the latest science and to publish them in a peer-reviewed format for broader distribution. Since 2018, a team of international scientists has worked together on such an update. The revised chapters are now published as a series of papers in this special section of the *Journal of Environmental Quality*.

2 | OVERVIEW OF KEY UPDATES

All of the original chapter topics are included in this special section: design (Clough et al., 2020), deployment (Charteris et al., 2020), air sample collection, storage, and analysis (Harvey et al., 2020), automated chambers (Grace et al., 2020), flux calculations (Venterea et al., 2020), and statistical considerations, emission factor estimation, and data reporting (de Klein et al., 2020). Because of their importance to minimize potential risks to researchers that use chamber methodologies, the H&S considerations are

included as an appendix to this introductory paper. In addition, two further topics are addressed as separate papers: N₂O modeling approaches (Giltrap et al., 2020) and guidelines for “gap-filling” missing measurements (Dorich et al., 2020).

Although this special section of the *Journal of Environmental Quality* presents a revision of earlier guidelines, basic guidance that was included in the first version is covered in the papers as well, to ensure that the reader is provided with one complete and comprehensive set of guidelines. All papers provide some refinement from the original chapters to include the latest literature and understanding of topics, but three chapters have undergone more extensive revision. The “deployment” paper (Charteris et al., 2020) now includes a more thorough discussion and analysis of the sources of variability associated with N₂O emissions. The “flux calculation” paper (Venterea et al., 2020) now includes a thorough analysis of different flux calculation (FC) methods and decision trees summarizing recommendations on procedures for screening data based on analytical error and minimum detectable fluxes, and for selecting the most appropriate FC method. The latter paper also provides supplemental information in the form of spreadsheets that perform site-specific error analyses and example calculations. Lastly, the “data reporting” paper (de Klein et al., 2020) has been expanded to include a review of recent approaches to statistical and meta-analysis of N₂O flux data and emission factors, and associated requirements for data reporting.

Each of the papers provides in-depth discussions of the current state of knowledge and defines minimum requirements for the various aspects of chamber methodologies. However, they are not meant to be highly prescriptive but instead aim to provide researchers with guidance on best practice and factors that need to be considered in the design and operation of N₂O experiments and measurement campaigns. The key findings of each paper are summarized in Section 3.

3 | SUMMARY OF MINIMUM REQUIREMENTS

3.1 | Chamber design

Clough et al. (2020) discuss minimum requirements for design features that affect N₂O determinations, including choice of materials, size, insulation, sealing, venting, depth of placement, and the need to maintain plant growth and activity. Current knowledge with respect to these factors is synthesized and discussed. For most of the chamber design features (e.g. materials, size, insulation, sealing between base and chamber, and venting), there seems to be good

scientific consensus on what the minimum requirements are. However, further systematic evaluation of fans is still required to determine best practice recommendations for their use inside chambers. Clough et al. (2020) also recommend that although chamber designs can be tailored to the ecosystem under study, the designs should be bench-tested to ensure that artifacts are prevented and the experimental objectives are met.

3.2 | Chamber deployment

Chamber deployment refers to how chambers are used to generate accurate and comprehensive flux datasets that, in conjunction with ancillary data, achieve the required experimental aims. This includes optimizing the experimental design and sampling strategy to account for the high spatial and temporal variability in N₂O fluxes and thus reduce the overall uncertainty of N₂O emission estimates. Charteris et al. (2020) provide a comprehensive set of recommendations on three key topics: (a) addressing spatial variability; (b) addressing temporal variability; and (c) practical and experimental aspects. The aspects relating to spatial variability include site selection, experimental design structure, chamber coverage and size, pre-experiment measurements to examine underlying flux variability, chamber placement to account for soil or crop features or gradients, and treatment application. Aspects of temporal variability include sampling after events that can stimulate emission (e.g., fertilizer application, rainfall, tillage), chamber closure time, time of day that best represents the daily mean emissions, sampling frequency through the experiment, and the duration of the experiment. Finally, recommendations relating to practical and experimental aspects of chamber deployment include chamber installation, sequencing and grouping of chamber measurements, enclosure period, the number of headspace samples, sampling at time zero, and the measurement of ancillary parameters to help interpret the N₂O flux data.

3.3 | Air sample collection, storage, and analysis

Procedures for air sample collection, storage, and analysis need to ensure that sample integrity is maintained during sampling and storage and that the analytical systems and detector options are set up correctly and are appropriately calibrated (Harvey et al., 2020). This paper describes optimal methods for collecting representative samples from the chamber headspace using syringes. Samples should be stored in small glass vials (≤ 12 ml) that are overpressurized

to reduce the risk of sample integrity loss. Sample analysis, most commonly using gas chromatography (GC), should be conducted within a few months of collection. Detailed discussion of the requirements for GC calibration is provided, including the use of standards and their traceability to enable accurate gas concentrations and fluxes to be derived. Although the electron capture detector (ECD) has been the detector of choice for GC analysis for decades, Harvey et al. (2020) discuss and summarize a number of alternative detectors that are increasingly being used. The detectors may offer some advantages (e.g., multiple gas species analyses), but they cannot always handle the relatively small sample volumes associated with vials. Some optical systems are well suited to continuous and switched-flow applications that are associated with autochamber systems.

3.4 | Automated chamber systems

The basic requirements of automated chamber design and deployment to minimize soil, plant, and environmental disturbance are identical to those for static chambers. Additional requirements and considerations include the ability to automatically open chambers when the outside environmental conditions change (e.g., temperature or rainfall), resilient design to reduce the risk of power failure and thus data loss, calibration of the analytical system, and automated or manual checks to minimize leaks and to ensure data quality (Grace et al., 2020). A major advantage of automated systems is that they allow for an increase in sampling frequency of the highly episodic N_2O emissions, thus improving the temporal integration of the fluxes and subsequently the accuracy of emission factor estimates.

3.5 | Flux calculations

The conversion of N_2O concentrations measured in individual chamber headspace samples to a corresponding flux value is another critical step in the overall methodology. It is well documented that different FC schemes can produce different flux estimates for a given set of chamber headspace data. The available FC schemes differ in their theoretical basis, computational requirements, and performance in terms of both accuracy and precision. Venterea et al. (2020) first review the essential theoretical and practical aspects of the most commonly used FC schemes and then provide recommendations for their selection and use. A gold standard approach is presented in the form of two decision trees: one for optimum selection of FC schemes depending on the availability of soil physical property data

and frequency of headspace sample collection during each chamber deployment, and one for identifying chamber data sets having fluxes below detectable levels or variances that reflect natural fluctuation in ambient N_2O concentrations. Both decision trees rely on information regarding the precision of the particular analytical system (e.g., gas chromatograph) used to determine N_2O concentrations in chamber headspace samples. Details regarding methods for determining analytical precision are provided, as well as methods for applying that information on a site-specific basis.

The paper by Venterea et al. (2020) is supplemented by several useful spreadsheets, providing examples for a wide range of commonly used calculations related to flux determination, as well as methods for applying Monte Carlo-based error analysis techniques for comparing the accuracy and precision of different FC schemes. The error analysis is based on user-supplied information regarding number of samples collected per chamber, chamber dimensions, deployment period, soil properties, and analytical measurement precision. Example error analyses are presented for hypothetical conditions illustrating how the analysis can be used to guide FC scheme selection, estimate bias, and inform design of chambers and sampling regimes.

3.6 | Statistical considerations, emission factor estimation, and data reporting

Statistical analysis of chamber data is challenged by the inherently heterogeneous nature of N_2O fluxes, so it is important that the analysis is sound and that emission factors are robustly estimated. de Klein et al. (2020) discuss methods for assessing normality and options for transforming data, including negative values, and review statistical approaches for analyzing N_2O data. This paper also provides minimum requirements for calculating emission factors from single experiments and provides guidance on reporting of (meta-)data from experiments to ensure that the reliability of the results can be assessed and emission factor values can be included in the emission factor database of the Intergovernmental Panel on Climate Change (IPCC, 2020). Data reporting should include key data on the experimental site (e.g., location, soil, and climatic conditions, and crop and management history), methodology (e.g., details on chamber design, treatments, trial design, sample frequency and duration, and gas analysis procedures including detection limits), data analysis (e.g., FC method, N_2O flux results per sampling day, uncertainty ranges of N_2O fluxes, and statistical analysis procedure), and finally results of key soil, climatic, crop, and management parameters.

3.7 | Nitrous oxide modeling and gap-filling procedures

As N₂O emissions are notoriously variable, frequent measurements and long-term campaigns are recommended to provide robust estimates of cumulative emissions. Due to practical and financial resource constraints, researchers are increasingly using modeling and gap-filling approaches to extrapolate field measurements to allow N₂O fluxes to be estimated when data are missing. Giltrap et al. (2020) review common modeling techniques such as calibration and validation, assessment of model fit, sensitivity analysis, and uncertainty assessment. They also summarize the examples of N₂O modeling for different purposes and describe some of the commonly used N₂O models. Dorich et al. (2020) provide an in-depth discussion on different gap-filling approaches and provide guidance on how to gap-fill N₂O datasets. These researchers describe five gap-filling practices (i.e., linear interpolation, generalized additive model, autoregressive integrated moving average, random forest, and neural networks) and provide strengths and weaknesses of each to aid the selection of the most appropriate gap-filling approach.

4 | BALANCING LIMITED RESOURCES

An important issue that emerged from discussions among contributors to the various papers was the difficulty of balancing competing demands with limited resources. In particular, the need to balance the benefits of increasing the number of chamber headspace samples, which increases the reliability of each individual flux measurement, against the benefits of increasing the number of chamber locations and/or sampling events to better capture the notoriously large spatial and temporal variability in N₂O fluxes. A key question underlying this debate is the acceptability of the assumption that the headspace N₂O concentration increases linearly during the enclosure period. Gas diffusion theory states that this increase will be nonlinear due to suppression of the vertical N₂O concentration gradient at the soil–atmosphere interface, which occurs as soon as the chamber is placed in position (Parkin, Venterea, & Hargreaves, 2012; Venterea, Spokas, & Baker, 2009). This further suggests that the N₂O flux at time zero (i.e., when the chamber is put in place, f_0) is the “true” flux that may be more accurately determined using a nonlinear FC method. Nonlinear FC methods, in general, require more headspace samples (at least four) to be collected per chamber measurement, compared with a FC based on linear interpolation. However, collecting more samples can put strains on financial resources and may limit the frequency of measurements required to better account for spatial or

temporal variability in N₂O emissions. Assessing the trade-offs between carefully determining individual flux measurements using a nonlinear FC method versus accepting a potential bias in a linearly estimated flux, while better capturing spatial and temporal variability, is a major challenge for researchers using NSS chamber methods. Any decisions on balancing limited resources to achieve the best possible (most accurate) results should, therefore, take into account the magnitude of the uncertainty associated with each step of the N₂O chamber methodology and the relative impact each of these uncertainties has on calculating cumulative emissions and emission factors. Two articles included in this special section discuss issues related to balancing resources in more detail and provide some tools for its assessment (Charteris et al., 2020; Venterea et al., 2020).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.


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APPENDIX: HEALTH AND SAFETY CONSIDERATIONS

The use of chambers to measure N₂O fluxes brings with it a number of H&S risks. It is important that research staff consider these prior to starting any chamber deployment and sampling. Researchers estimating the resource requirements of any chamber experiment should budget for H&S considerations—for example, the number of people required to safely collect samples from chambers. Table A1 provides an overview of the major H&S issues for each stage of N₂O chamber methodologies. The information provided here is not intended as comprehensive. Although local site (field and laboratory) conditions should also be taken into account, the issues discussed (Table A1) should be considered as a minimum when complying with institutional and national legislation, and hazardous substance procedures. Finally, staff should be encouraged to report all accidents and “near misses” associated with chamber methodologies to appropriate H&S officers and/or committees of their institution. In this way, systematic accidents can be identified and procedures put into place before there is any major problem. This reduces future risks to all workers.

TABLE A1 A summary of potential health and safety (H&S) risks associated with chamber methodology, and guidelines on how to reduce them

Stage	Risk	Consideration
Chamber design	Cuts, lacerations from sharp edges	Construction material and final design should be selected to minimize sharp edges.
	Fumes from glues used to bond chamber sides	Any gluing should be conducted in well-aerated rooms, or outdoors. Consider wearing a facemask.
	Manual handling—muscle strain, back problems, crush injuries	Bulky and/or heavy chambers should be lifted between at least two people or by machine. Gloves and protective footwear (hard boots) should be worn.
Chamber deployment	Manual handling—muscle strain from installing multiple chambers, crush injuries from using hammers, and lacerations from using sharp implements during chamber installation	Gloves and hard boots should be worn to avoid injury to hands and feet from hammers and sharp implements when installing chamber bases. Workload should be shared between people to avoid one individual overstraining muscles and joints when installing multiple chambers.
Sample collection, storage, and preparation	Muscle strain from repetitive actions, such as bending and use of syringes	Workers should avoid rushing by giving themselves sufficient time between sampling multiple chambers. Workloads must be shared. Job rotation should minimize impacts. Chamber design and sampling approach should be considered to minimize the muscular effort required for repeated sampling—the size of needle used can affect the effort required to fill a syringe, for example. Perhaps set a maximum number of chambers one person can sample per day.
	Fatigue	Avoid overly long field sampling campaigns that require driving to site(s). Take adequate breaks and rest if feeling fatigued.
	Needle-stick injuries	Workers should take care when using exposed needles in the field on uneven, sometimes slippery surfaces. When not in use, needles should be in guards at all times. New needles should be used at each sampling occasion to minimize infections from a needle-stick. Workers should leave sufficient time between sampling multiple chambers to avoid rushing. Needles should ideally be thrown away after each sampling and definitely after a needle-stick. All needles, syringes, and vials should be carefully removed from the field site after each sampling to avoid future injuries from debris left behind.
	Personal protective equipment and exposure to sun and cold weather	Workers should take appropriate precautions to avoid sunburn—by applying sunscreen, wearing a hat and long sleeves—and heat exhaustion. Take plenty of water. Workers should wear sufficient clothing and waterproof footwear to keep warm and dry in cold and/or wet weather.
	Exposure to microbiological agents when dealing with livestock feces	Where appropriate, personal protective equipment such as gloves, overalls, and face masks should be worn. Any open cuts to the skin should be covered before going into the field. Ensure thorough hand washing when finished, especially before eating, drinking, and smoking.
	Exposure to chemicals	Researchers should read the material safety data sheets of chemical products such as fertilizers and inhibitors before using them in the field. Appropriate personal protective equipment should be used.
	Lone field working	Working alone cannot always be avoided. Wherever possible, more than one staff member should sample. Where this is impractical, the field worker or researcher should adhere to the “working alone” policy of their institution. If such a policy does not exist, ensure someone in their institution knows that they are safe, such as by scheduled phone calls. The lone worker should take a mobile phone into the field and ensure that it has signal.

(Continues)

TABLE A1 (Continued)

Stage	Risk	Consideration
Automated systems	Electrical supplies	Preferably, all field electrical supplies should be low voltage. Main voltage supplies must be isolated, or protected by residual current devices, in accordance with legislation.
	Crushing injuries (moving parts)	Workers should be made aware of moving parts capable of crushing hands, fingers, etc. Where appropriate, these moving parts should have guards.
	Manual handling	Gloves and hard boots should be worn to avoid injury to hands and feet when using hammers and sharp implements when installing chambers. Workload should be shared between people, to avoid one individual overstraining muscles and joints when installing multiple chambers.
	Trip hazards	Gas lines and electrical cables should be tidied and arranged—in bundles where possible—to minimize potential trip hazards.
	Lone field working	Working alone cannot always be avoided. Wherever possible, more than one staff member should sample. Where this is impractical, the field worker or researcher should set up procedures to ensure someone in their institution knows that they are safe, such as by scheduled phone calls. The lone worker should take a mobile phone into the field and ensure that it has signal.
Laboratory sample analysis	Manual handling, e.g., gas cylinders	Where appropriate, use cylinder trolleys and lifts to move gas cylinders. Wear protective footwear.
	Compressed gases, pressure or vacuum; noise	Train operators in safe use of compressed gases, (includes regulators, changing cylinders, cylinder clamps and holders). Good ventilation is essential. Use ear and eye protection where required.
	Chemical exposure	Use appropriate control measures where chemicals are used, or gas chromatography laboratories are shared within larger chemistry laboratories. Wear laboratory coats and disposable gloves if exposed to chemicals.
	Ergonomic strain	Back problems from standing all day: use specialized laboratory chairs, and perhaps use anti-fatigue matting.
	Needle-sticks	Workers should take care when using exposed needles. The laboratory environment has more stable walking surfaces than does the field but can sometimes be slippery. When not in use, needles should be in guards at all times. New needles should be used each day, to minimize infections from a needle-stick. Workers should avoid rushing. All used needles, and any from a needle-stick, should be carefully disposed of in a suitable sharps bin.
Data analysis	⁶³ Ni-ECD (electron capture detector) operation (radioactive source)	“Wipe test” and disposal procedures (e.g., testing for radioactive leakage from the sealed source in the detector) should be conducted in accordance with the manufacturer’s and regulatory authority requirements.
	Muscle strain and repetitive strain injury (RSI)	Ergonomic impact (RSI) from repetitive actions is a risk, especially in data manipulation. The main precaution is to break work up into manageable chunks, with rest breaks and a chance for different activities throughout the day. Ergonomic mouse and keyboard can be used.
	Monitor glare	Main controls are antiglare screens, and taking regular breaks. Keep up to date with optician eye checks.
	Muscle strain and repetitive strain injury (RSI)	Ergonomic impact (RSI) from repetitive actions is a risk, especially in data manipulation. The main precaution is to ensure an appropriate setup of computer workstations and consider the use of ergonomic mouse and keyboard. Furthermore, data analysis work needs to be broken up into manageable chunks, with rest breaks and a chance for different activities throughout the day.
	Monitor glare	Main controls are antiglare screens, and taking regular breaks. Keep up to date with optician eye checks.